Intersectional Hybrids in Isoëtes[1]

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Engelmann (1886) and Campbell (1891) have described the simple procedure for germinating *Isoëtes* spores in the laboratory to obtain micro- and megagametophytes. Perhaps the absence of hybridization studies in this genus can be explained partly by the fact that sexually mature living plants of more than one species were rarely assembled at one time, or that when they were, the primary aim was comparative embryology (La Motte, 1937). The present study was undertaken to test for genetic compatibility among four selected *Isoëtes* species in three sections of the genus (*sensu* Pfeiffer, 1922). All crossing combinations produced progeny, and in at least one species apogamy may occur.

MATERIALS AND METHODS

Spores for the crosses were obtained from populations of plants as follows: *Isoëtes (Reticulatae) macrospora* Dur.—Monroe Co., TN, *Boom 318,* Shenandoah Co., VA, 8 Nov 1978, *Evans; I. (Reticulatae) engelmannii* A. Br.—Polk Co., TN, *Boom 317*, Putnam Co., TN, *Boom 267; I. (Tuberculatae) flaccida* Shuttlew.—Dixie Co., FL, *Boom 313,* Wakulla Co., FL, *Boom 314, 315; I. (Cristatae) riparia* Engelm.—Tyrrell Co., NC, *Boom 316.* Voucher specimens have been deposited in the Herbarium of the University of Tennessee (TENN). All plants were collected during the summer or autumn of 1978, and were grown in the greenhouse for a short time until the crosses were made in mid-January, 1979.

The crossing technique was quite simple, yet rigorously controlled. Forty-eight glass vials were filled with about 1 ml of sterilized fine sand and 10 ml of sterilized pond water. Each sporangium was dissected out of the sporophyll base, washed in a sterile water bath, and then teased apart to release the spores into a vial, taking great care to insure that the vials were not contaminated with unwanted spores. Since microsporangia and megasporangia are usually found on the same plant, spores were taken from completely intact sporangia to avoid the possibility of using megagametophytes which already had been fertilized.

The crosses among the eight populations were set up in such a way that the megaspores of each population were brought into contact with the microspores of every other population. To test for spore viability and self compatibility, one plant from each population was selfed. To provide controls for the crosses and to test for apogamy, one vial was set aside for each population in which only megaspores were placed. All vials were kept in the greenhouse at about 25° C, where they were exposed to normal ambient light fluctuations, and were not disturbed except for the occasional addition of sterile pond water.

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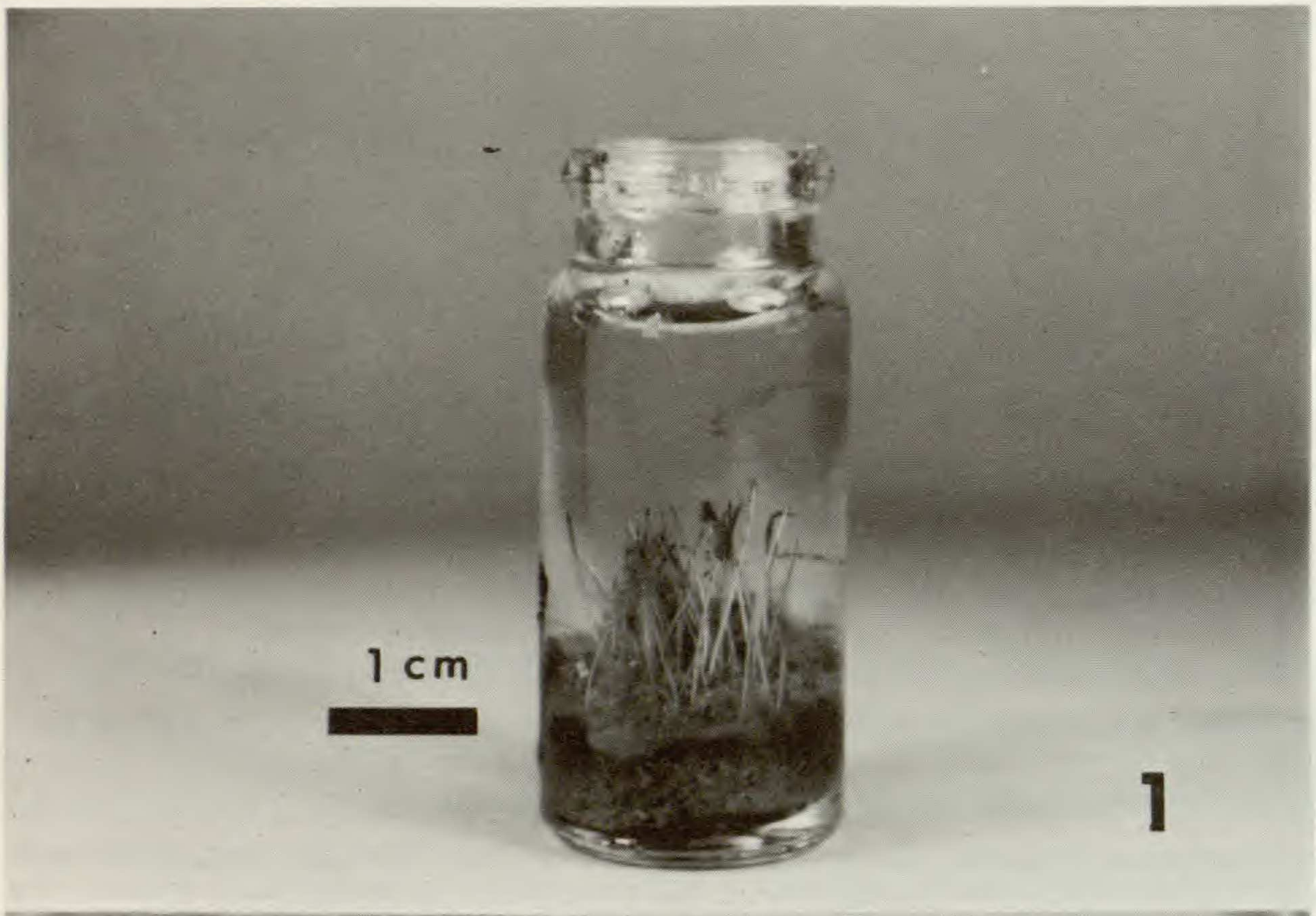

FIGS. 1–2. Sporophytes resulting from a cross between *Isoëtes flaccida* and *I. macrospora*, two species thought to have had very different evolutionary histories. FIG. 1. Sporophytes in vial at two months after the cross was made. FIG. 2. Hybrid sporophytes at four months.

RESULTS AND DISCUSSION

About seven weeks after the crosses were made, the first green shoots of young *Isoëtes* sporophytes were observed in a number of the vials (*Figs. 1* and *2*). Although not every individual cross was successful, every possible crossing combination was successful within the first two months of the experiment. None of the control vials showed any signs of growth at that time, and the genetic compatibility of the four species presumedly has been demonstrated.

No new sporophytes were observed until 3.5 months after the experiment began, when a single sporophyte appeared in the *I. macrospora* control vial (*Boom 318*). Since no microspores had been introduced into this vial, either the megagametophyte had somehow become fertilized before it was introduced into the vial or the species is capable of reproducing apogamously. Considering the precautions taken by using only fully intact sporangia as a source of spores, apogamy seems more likely. Eight months after the crosses were made, no sporophytes had developed in any of the control vials of the other three species.

Easily hybridized species of *Isoëtes* means that hybridization followed by polyploidization may be a mode of evolution from time to time in the genus. The occurrence of facultatively apogamous taxa is consistent with such a process. If, as is suggested by the experimental observations, *I. macrospora* can be apogamous, this could help explain the Virginia and Tennessee populations disjunct from the typical northeastern range of this species (Dennis et. al., 1979). The reticulate distal face and the cristate proximal face of the megaspores of *I. macrospora* suggest a possible hybrid origin for this species.

Some *Isoëtes* populations on the Coastal Plain of the Carolinas have various characters, primarily megaspore ornamentation, which clearly are intermediate between typical *I. engelmannii* and *I. riparia*; supposedly these plants are hybrids between the two. Specimens from such populations occasionally have been annotated as *I. engelmannii* var. *georgiana* Engelm. or var. *caroliniana* Eaton.

The results of this study also support Matthews and Murdy's (1969) interpretation of the often confusing *Isoëtes* populations on the granite outcrops of the Piedmont of the southeastern United States. Introgression apparently is taking place in pools which are ecologically intermediate between the habitats typical of *I. piedmontana* (Pfeiffer) Reed and those of *I. melanospora* Engelm. For an alternate explanation, see Rury (1978), who suggests that intermediates represent developmental stages of one polymorphic species.

The naturalness of Pfeiffer's (1922) sections of the genus is suspect now more than ever in light of the artificial intersectional hybridizations. The infrageneric classification of *Isoëtes* should be reexamined by means of an extensive genetic, cytogenetic, and phytochemical survey, as well as by using traditional morphological characters.

This report of intersectional genetic compatibility need not necessarily affect *Isoëtes* taxonomy at the species level, however. In natural circumstances, the taxa generally are isolated by geographic, ecological, or phenological barriers, and they can be distinguished morphologically from one another. The amount of gene

flow between the typically isolated populations must be relatively small. If this is not the case, it remains a challenge to explain why selection has not favored the establishment of reproductive barriers between species.

The present study was initiated to test the potential for genetic experimentation in *Isoëtes*. The preliminary results were very successful and indicate further and wider genetic studies would be beneficial. Such future hybridization research should take advantage of the artificial crossing technique recently described for *Selaginella* (Webster, 1979). The method appears to be well suited for *Isoëtes* crossing with little or no modification, and will permit more critical experimentation than ever could be possible with the non-sterile technique employed in the present study.

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LITERATURE CITED

CAMPBELL, D. H. 1891. Contributions to the life-history of Isoëtes. Ann. Bot. 5:231–358.

DENNIS, W. M., A. M. EVANS, and B. E. WOFFORD. 1979. Disjunct populations of Isoëtes macrospora in southeastern Tennessee. Amer. Fern J. 69:97–99.

ENGELMANN, G. 1886. The genus Isoëtes in North America. Trans. St. Louis Acad. Sci. 4:358–390.

LA MOTTE, C. 1937. Morphology and orientation of the embryo of Isoëtes. Ann. Bot. 1:695–715.

MATTHEWS, J. F. and W. H. MURDY. 1969. A study of Isoëtes common to the granite outcrops of the southeastern piedmont, United States. Bot. Gaz. 130:53–61.

PFEIFFER, N. E. 1922. Monograph of the Isoëtaceae. Ann. Mo. Bot. Gard. 9:79–232.

RURY, P. M. 1978. A new and unique, mat-forming Merlin's-grass (Isoëtes) from Georgia. Amer. Fern J. 68:99–108.

WEBSTER, T. R. 1979. An artificial crossing technique for Selaginella. Amer. Fern J. 69:9–13.

REVIEW

"THE ECONOMIC USES AND ASSOCIATED FOLKLORE OF FERNS AND FERN ALLIES," by Lenore Wile May, Botanical Review 44:491–528. 1979—As stated by the author, this paper is not taxonomic in nature, but discusses fern folklore and to a lesser extent their economic history. It provides an easily read text for the generalist and a good bibliography for those persons interested in pursuing this topic further. Some of the section titles include: Folklore, Fern Dyes, Fern Fibers, Fern Foods, Medicinal Uses of Ferns, The Male Fern, and The Bracken Fern. The section on medicinal uses occupies forty percent of this article, with related medicinal notes in the folklore portion. The author mentions the following about *Ophioglossum vulgatum:* "This plant is called adder's tongue because out of every leaf it sendith forth a kind of pedestal like an adder's tongue, it cureth the biting of serpents."—*J. Scott Peterson, Dept. of Botany & Plant Pathology, Colorado State University, Ft. Collins, CO 80523.*